ALLOMETRY IN NORMAL AND REGENERATING ANTENNAL SEGMENTS IN DAPHNIA

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The growth of a part in relation to that of the whole body in many animals follows the law of allometry and may be expressed by the equation

$$y = bx^{\alpha}$$

where y is the size of the part; x, the size of the whole; and b and α are constants (Huxley, 1932 and Huxley and Teissier, 1936). Regeneration has been " regarded as an acceleration of normal growth processes " (Przibram, 1919 and 1926). Regenerative growth might therefore also be expected to follow the law of allometry. Using the data of Zeleny (1908) on the gulf-weed crab, *Portunus sayi*, Huxley (1931) demonstrated that the above equation holds for the size of the chela in relation to the body as a whole and also for the amount of regeneration of the chela during any one instar in relation to the size of the animal during that instar. More recently others, especially Paulian (1938), have shown that in many arthropods the amount of regeneration of a part during any one instar in relation to the size of the body follows the law of allometry. Paulian has also pointed out that normal and regenerating antennae in *Gammarus pulex* and in *Carausius morosus* increase exponentially with time. Inasmuch as the relative growth equation is derivable on the assumption that the parts increase exponentially with time (Huxley, 1932 and Lumer, 1937), we may conclude that regeneration of the antennae in *Gammarus pulex* and in *Carausius morosus* also follows the law of allometry.

In the above cases we are dealing with regeneration that tends to be complete. Do these relations hold in a form where regeneration is not complete? In seeking a solution to the problem *Daphnia magna* is well suited as an experimental animal. After amputation of an antenna regeneration is limited to the restoration of the most proximal segment injured and the formation of setae. The amount of regeneration varies with the level of injury within the segment (Anderson, 1935).

The present study is a determination of the relations of the growth of normal and regenerating antennal segments to that of the animal as a whole in *Daphnia magna*.

Experimental Procedure

Females from a single clone of *Daphnia magna* Straus were used. The culture medium was pond water rich in organic matter. Individuals were isolated within six hours after their release from the mothers and placed in watch glasses with a few drops of culture medium. Just enough of a saturated solution of chloretone was added to make the animals immobile. Each animal was placed on its left side with the left antenna stretched out in front of the body. Those animals that were used to study the growth of normal segments were then drawn with the aid of camera lucida. After the drawings were made the animals were placed in individual vials containing about sixty cubic centimeters of fresh culture medium. In the case of those animals that

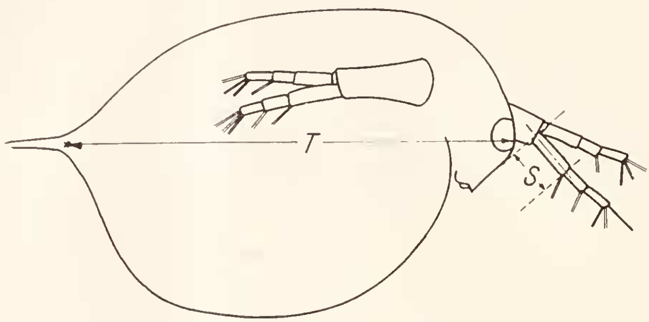

Fig. 1. Diagram showing the method of making measurements. *T*—total length, longest dimension of the body exclusive of the spine. *S*—segment length taken on the central axis of the segment.

were used to study regeneration, the first segment of the ventral ramus of the left antenna was severed by applying pressure with a needle which had been ground to a chisel edge. The level of amputation was varied in each instance. After the operation these animals were also placed in individual vials containing fresh culture medium. Several hours later the operated animals were again placed in watch glasses and immobilized with chloretone. They were then drawn in the same manner as were the unoperated animals. Care was taken to denote exactly the extent of the brown area just proximal to the level of amputation, for this is injured tissue that is cast off at the next molt (Anderson, 1935). After being drawn, they were replaced in their respective vials. From this point on both normal and operated animals were treated alike. Each animal was drawn during each successive instar up to and including the tenth. Inasmuch as the animals change in size only at ecdysis and immediately thereafter (Agar, 1930), drawings were made at any time during the instar. Every time after an animal was drawn it was placed in fresh culture medium. The experiment was run at room temperature (18°–26° C.).

Measurements of the total length of the animals and the length of the antennal segments were made from the drawings. The total length of the animal was taken as the distance from the base of the spine to the most anterior point on the head. The length of the antennal segment was taken on the central axis of the segment. These measurements are illustrated in Fig. 1, and conform to those made by Anderson (1932, 1935) in other studies on *Daphnia magna*.

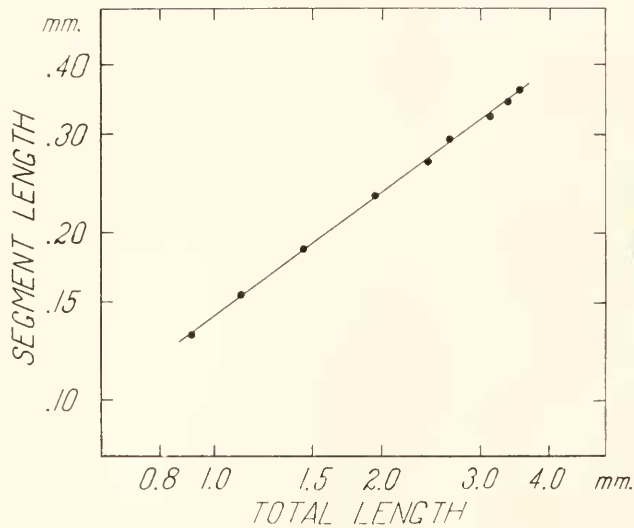

FIG. 2. Double logarithmic plot of the relations between the lengths of the normal segment and the total lengths during each of the first nine instars.

RELATIVE GROWTH OF THE NORMAL ANTENNAL SEGMENT

The relation of the logarithms of the mean lengths of the first segment of the ventral ramus of the left antenna to the logarithms of the mean total lengths of eighteen animals for the first nine instars is shown in Fig. 2. The points fall approximately in a straight line. We may therefore conclude that the law of allometry holds and the relation between the length of the segment and the total length may be expressed by the equation

$$y = bx^{\alpha} \tag{1}$$

where y is the length of the segment and x is the total length of the animal. The values of the constants b and α are 0.145 and 0.74, respectively. These were determined by the method of least squares. The value of α being less than unity indicates that the antennal segment grows at a lower rate than the body as represented by the total length. The antenna as a whole also grows more slowly than does the body as

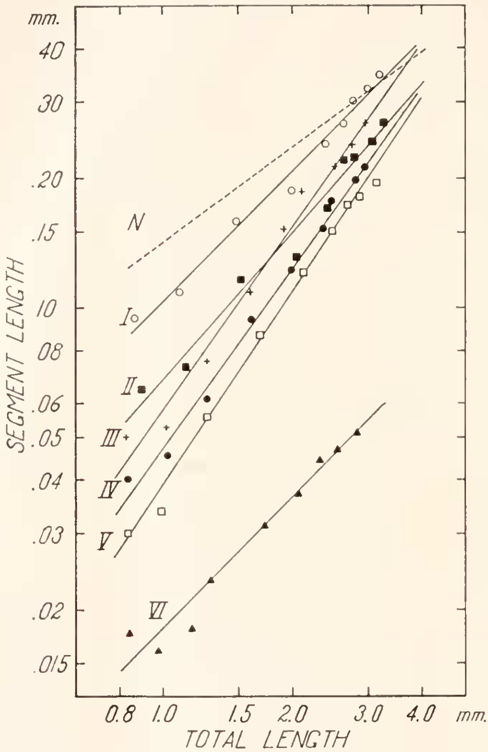

FIG. 3. Double logarithmic plots of the relations between the lengths of regenerating segments and the total lengths during each of the first nine instars for animals with antennae amputated at different levels. The level of injury is designated by the Roman numerals whose values are given in Table I. The dash line designated by N is that for the normal segments shown in Fig. 2.

is evidenced by the fact that the young at birth have antennae whose length is greater in proportion to the body than do the adults. The antennal segments, however, maintain the same proportions to each other throughout life.

Huxley (1931) has shown that the limbs of sheep grow less rapidly than the body after birth. This is also true for the macaques (Lumer and Schultz, 1941) and probably for all mammals where the young at birth are able to run along with their mothers and perhaps for many other animals that depend on their means of locomotion for protection and/or food-getting.

Regeneration

The nature of regenerated antennae has been adequately described by others and need not be repeated here. Regeneration is limited to the restoration of the most proximal segment injured and the formation of new setae (see Anderson, 1935). The amount of regeneration is quantitatively related to the level of injury within the segment.

The operated animals were divided into six classes on the basis of the length of the intact portion of the segment during the latter part of the instar of amputation. The relations of the logarithms of the mean

Table I

The values of b and α for the relations of the length of the regenerating first segment of the ventral ramus of the left antenna to the total length of the animal.

Class	Level of Injury *	Number of Cases	b	α
I	0.076–0.110	8	0.103	1.01
II	0.060–0.070	7	0.068	1.14
III	0.046–0.053	7	0.058	1.42
IV	0.040–0.042	8	0.047	1.40
V	0.027–0.030	9	0.039	1.49
VI	0.010–0.023	7	0.018	1.02

* Length of the intact portion of the segment in millimeters during the instar of amputation. The values of b and α were determined by the method of least squares.

lengths of the amputated segments to the logarithms of the mean total length of the animals for the instar of amputation and the next eight instars for each class are shown in Fig. 3. While the points for any one class do not fall along a straight line as closely as do those for the relations in unoperated animals (Fig. 2), they do approximate a straight line. The law of allometry may be considered applicable and the relations can be expressed by the equation (1). The values of the constants are given in Table I.

The value of the constant α in the equation (1) is the ratio of the percentage increase of y to the percentage increase in x. Since x always represents the total length of the animals, both normal and operated, in the relations described above, the values of α are directly comparable. Examination of Fig. 3 and Table I shows that the value of α increases as the level of injury reaches a lower point in the segment until a certain level is reached after which the value of α decreases. This relation is brought out graphically in Fig. 4. Another point worthy of note is that in Fig. 3 the curves with one exception tend to converge at a point where the total length would be about five millimeters, the maximum size that the animals reach. From this it is apparent that as long as the level of injury is above the critical level, the growth rate of the regenerating antennal segments is such that they approach the length of the normal segment simultaneously as full growth of the animals is attained.

Somewhat analogous results have been found by others. Zeleny (1905, 1909) found that the rate of regeneration of an organ in many

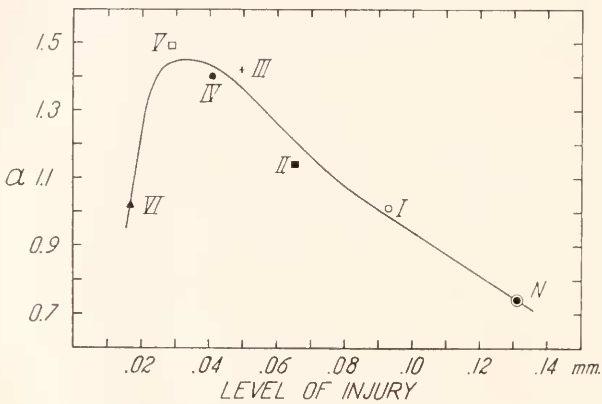

FIG. 4. The relation between the value of α in the equation

$$y = bx^{\alpha}$$

and the level of injury. The symbols correspond to those used in Fig. 3.

animals increases with the degree of injury up to an optimum, after which the rate decreases. Zeleny's work is not directly comparable inasmuch as he was concerned with the rate of regeneration of an organ when that one only was removed in comparison with the rate when several others were removed in addition. The results of Paulian (1938) are more directly comparable. He amputated the antennae of *Gammarus pulex* and *Carausius morosus,* and inspection of his figures (Figs. 14 and 15, pages 320 and 322) indicates that the rate of growth of the antennae increased as the level of amputation approached the proximal end. Whether or not a critical level might be reached beyond which the rate decreases, was not determined in his experiments. Further, the amputated antennae reach the size of the normal at different times, the time taken varies directly with the amount removed.

The Significance of the Constant b

The question of the biological significance of the constants b and α in the law of allometry have been subject to considerable discussion. Huxley (1932) and Needham (1934) have stated that the constant b is of little biological importance. The value of the constant b is that of y when $x = 1$. As a consequence, its value changes with the unit of measure employed while the actual relations of y and x remain the same. Again the unit chosen is usually such that b is an extrapolated value of y. Because of these arbitrary factors the significance of b has remained elusive. Recently Lumer, Anderson, and Hersh (1941) have pointed out that if b is to have biological significance, the unit of measure chosen should be one given by the organism. They suggest that the most satisfactory unit would be the size of a standard part at the beginning of a developmental period, but where this cannot readily be ascertained an approach to it could be made by taking the smallest value of the standard part given by the data as unity. In this way b would be an actual value of y. This is in line with the proposal of Huxley and Teissier (1936) that b should be called the "initial-growth index," for indeed that is what it becomes as far as the data are concerned when the above suggestions are followed. The constant α has presented no such difficulties. Since it is the ratio of the percentage growth rates of the parts y and x, it is constant regardless of the unit of measure used. α has therefore been considered of relatively greater importance than b.

Lumer, Anderson, and Hersh (1941) have shown how the constant b may be made a more tangible entity in that it can be given in terms of the organism. As such it has significance. The question still remains as to the degree of its importance. If the value of b, i.e., the initial ratio of y to x, could be altered experimentally, and if as a consequence the value of α would change, we could conclude that the value of b determines the value of α; b would then have a greater biological importance than heretofore supposed.

This is precisely what we have done in the experiments described in this paper. We have amputated the antenna and so reduced the length of the segment. The ratio of the intact portion of the segment during the instar of amputation to the total length of the animal is given by b, since the total length during that instar is approximately one millimeter and the millimeter is the unit of measure. Following amputation, the growth rate of the antennal segment is changed so that new values of α result. Further, as b decreases α increases until b reaches a particular value, after which α also decreases as is shown in Table I and Fig. 4. The constant b, in the sense in which we have employed it, serves as a measure of the conditions at the beginning of the developmental period, and as these conditions differ, so also do the consequent rates of development as represented by the constant α.

Summary

The law of allometry

$$y = bx^{\alpha}$$

was found to be applicable to both normal and regenerating antennal segments in *Daphnia magna*.

The growth rate of the regenerating segments increases as the level of injury approaches the proximal end of the segment until a critical point is reached, after which the rate decreases. As long as the level of injury is distal to the critical level, the growth rate is such that the regenerating segments tend to approach the length of the normal segment simultaneously as full growth of the animals is attained.

The significance of the constant b in the law of allometry is discussed.

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